ART 34 AMDT

CLAIMS

1. A method of nuclear transfer, comprising selecting and segregating G1 cells from a proliferating or non-proliferating population of diploid donor cells and transferring a nucleus from such a segregated G1 cell into an enucleated recipient cell, wherein donor cells are selected and segregated by physical picking based on individual cell identification to produce a pure G1 cell population with the proviso that said diploid donor cells are not selected from blastomeres which have been synchronised by ≥5μM nocodazole or 5 μg/ml colcemid.

2. A method as claimed in claim 1, wherein the donor cell population is at one or more known or unknown stages of the cell cycle.

3. A method as claimed in claim 1 or 2, wherein said donor cell population is non-proliferating and has been synchronised at any point in the G1 stage of the cell cycle.

4. A method as claimed in any one of claims 1 to 3 wherein said G1 cell is segregated at an early G1 phase.

5. A method as claimed in any one of claims 1 to 3, wherein the donor cell population is non-proliferating and comprises senescent cells.

6. A method as claimed in any one of claims 1 to 5, wherein said donor cell population is derived from either embryo, fetal, juvenile or adult cells isolated from an animal *in vivo* or from a cell culture *in vitro*.

7. A method as claimed in claim 6, wherein said donor cell population comprises any diploid karyotypically normal cell capable of being stimulated to enter the cell cycle and proliferate.

8. A method as claimed in claim 7, wherein said donor cell population is of an undifferentiated cellular state or are at any degree of differentiation or quiescence or senescence.

AMENDED SHEET
IPEA/AU

ART 34 AMDT

9. A method as claimed in any preceding claim wherein the donor cells are adult or fetal fibroblasts or follicular cells.

10. A method as claimed in any preceding claim wherein said donor cells comprise genetically modified cells.

11. A method as claimed in claim 10 wherein said donor cells comprise transgenic cells.

12. A method as claimed in any preceding claim, ~~wherein the recipient cell comprises~~ an enucleated oocyte.

13. A method as claimed in claim 12, wherein the enucleated oocyte is obtained from a species corresponding in origin to the donor nuclei.

14. A method as claimed in any one of claims 1 to 11, ~~wherein the recipient cell~~ comprises an enucleated stem cell or a clump of enucleated stem cells fused together.

15. A method as claimed in claim 14, wherein the stem cells are embryonic stem cells isolated from a growing embryo or form an established cell line in culture.

16. A method of producing cloned animal embryos which comprises transferring a diploid donor nucleus from a cell selected and segregated in the G1 stage of the cell cycle according to claim 1 into an enucleated recipient cell, with the proviso that said diploid donor cells are not selected from blastomeres which have been synchronised at G1-phase by ≥5μM nocodazole or 5 μg/ml colcemid.

17. A method as claimed in claim 16, wherein the donor nuclei are genetically altered using methods well known in the art to produce cloned embryos having desirable genetic traits.

AMENDED SHEET
IPEA/AU

18. A method as claimed in claim 16 or 17, when used to produce an animal species of cloned embryo selected from the group comprising birds, amphibia, fish and mammals.

19. A method as claimed in claim 18, wherein said cloned animal embryo is a mammal, selected from the group comprising primates including humans, rodents, rabbits, cats, dogs, horses, cattle, sheep, deer, goats and pigs.

20. A reconstituted animal embryo prepared by the method claimed in claim 16.

21. A reconstituted animal embryo as claimed in claim 17, comprising a transgenic embryo.

22. A reconstituted animal embryo as claimed in claim 20 or 21 re-cloned to further increase embryo numbers or which undergoes serial nuclear transfer to aid nuclear reprogramming and/or development.

23. A reconstituted animal embryo as claimed in any one of claims 20 to 22, comprising a species of mammal selected from the group comprising primates including humans, rodents, rabbits, cats, dogs, horses, cattle, sheep, deer, goats and pigs.

24. A method of cloning a non-human animal comprising the steps: (1) producing a cloned non-human animal embryo according to the method of any one of claim 16 or 17 (2) allowing a non-human animal to develop to term from the embryo by known methods; and (3) optionally breeding from the non-human animal so formed either by conventional methods or by further cloning.

25. A method as claimed in claim 24, wherein said cloned non-human animal is a non-human mammal selected from the group comprising non-human primates, rodents, rabbits, cats, dogs, horses, cattle, sheep, and deer.

26. A method as claimed in claim 24 or 25, wherein said cloned non-human animal is a transgenic non-human animal having a desirable genetic trait.

27. A method as claimed in claim 26, wherein said transgenic non-human animal is a transgenic bovine or ovine.

28. A cloned non-human animal prepared by the method of claim 24.

29. A cloned non-human animal as claimed in claim 28 comprising a mammal selected from the group comprising non-human primates, rodents, rabbits, cats, dogs, horses, cattle, sheep, and deer.

30. A cloned non-human animal as claimed in claim 28 or 29 comprising a transgenic non-human animal having a desirable genetic trait.

31. A cloned non-human animal as claimed in claim 30 comprising a transgenic bovine or ovine.

32. A cloned transgenic animal as claimed in claim 30 or 31 wherein the desirable genetic trait is selected from the insertion, deletion, or modification of a gene or genes enabling the production of pharmaceutical proteins in milk, blood or urine; production of nutraceutical products in milk or meat; production of beneficial agricultural traits to improve the quality of milk, meat and fibre production; improve resistance to pests and diseases; production of industrial proteins in milk; xenotransplantation; and for the generation of transgenic animals as models for human disease.

33. Offspring and descendants of the cloned non-human animal as claimed in any one of claims 28 to 32.

ART 34 AMDT

34. A method of producing a cell line comprising the steps a) selecting and segregating G1 cells from a proliferating population of diploid donor cells or from a synchronised population of diploid G1 cells or from a population of diploid senescent cells, and transferring a nucleus from such a segregated cell into an enucleated recipient cell; b) optionally growing to embryo stage; c) recovering cells; and d) establishing an immortalised cell line *in vitro* by methods known in the art.

35. A method as claimed in claim 34, wherein said cell line is an embryonic stem cell line.

36. A method as claimed in claim 34 or 35, wherein said donor cells are human cells.

37. A method as claimed in any one of claims 34 to 36, wherein both donor and recipient cells are human cells.

38. A method as claimed in any one of claims 34 to 37, wherein the donor cells are adult or fetal cells selected from any karyotypically normal cell type and the recipient cells are selected from any cell type capable of reprogramming gene expression.

39. An embryonic cell line produced by the method of any one of claims 34 to 36.

40. A human embryonic stem cell line produced by the method of claim 36, when dependent upon claim 35, useful in therapeutic applications.

41. A method of producing stem cells comprising the steps of a) selecting and segregating G1 cells from a proliferating population of diploid donor cells or from synchronised population of diploid G1 cells or from a population of diploid senescent cells and transferring a nucleus from such a segregated cell into an enucleated recipient cell; b) optionally growing to embryo stage; and c) recovering stem cells.

ART 34 AMDT

42. A method as claimed in claim 41, wherein said stem cells are embryonic stem cells.

Sub A13

43. A method as claimed in claim 41 or 42, wherein said donor cells are human cells.

44. A method as claimed in any one of claims 41 to 43, wherein both donor and recipient cells are human cells.

45. A method as claimed in any one of claims 41 to 44, wherein the donor cells are adult or fetal cells selected from any karyotypically normal cell type and the recipient cells are selected from any cell type capable of reprogramming gene expression.

46. Embryonic stem cells produced by the method of any one of claims 42 to 44.

47. Embryonic stem cells as claimed in claim 46, comprising human embryonic stem cells.

48. A use of the embryonic cells of any one of claims 39, 40 and 46, wherein specialised types of cell or tissue selected from the group comprising nerve cells, muscle cells, heart cells, liver cells, lung cells, kidney cells or any other type of cell of interest are cultured using methods well known in the art.

Sub A14

49. A use as claimed in claim 48, wherein said embryonic cells are human embryonic stem cells as claimed in claim 40 or 47.

50. A method of therapeutic cloning, wherein embryonic stem cells are produced according to any one of claims 35 and 41 to 44 from a donor cell derived from a subject, and cultured to produce specialised cells or tissue for transplantation in said subject or in another subject in need of such treatment.

AMENDED SHEET
IPEA/AU

51. A method as claimed in claim 50, wherein said embryonic stem cells comprise one or more transgenes to confer a desirable genetic trait in the resulting differentiated cells used for transplantation.

52. A method of treating a disease, disorder or injury which may be treated by transplantation of specialised cells or tissue, comprising administering to a patient in need thereof a therapeutically effective amount of specialised cells or tissue produced according to the method of claim 50 or 51.

53. A method as claimed in claim 50 or 51, wherein said disease, disorder or injury is selected from various neurological disorders (*eg* Parkinson's disease), diabetes, heart disease, muscular dystrophy, various hereditary diseases, specific cancers (*eg* leukemia), spinal cord injury, burns and other afflictions.

54. A method of drug discovery or toxicology testing of drugs using *in vitro* differentiated human embryonic stem cells produced by the methods of claim 48.

55. A method of xenotransplantation, wherein cells, tissues and organs are isolated from the non-human cloned animal of any one of claims 28 to 32, and used for transplantation in a human patient in need thereof.

56. A method of gene therapy, wherein cells, tissues and organs comprise a transgene and are isolated for the non-human cloned animal of claim 30 or 31.